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Morphology and Asexual Reproduction of *Vitreochlamys aulata* (Volvocales, Chlorophyceae)

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The vegetative morphology and asexual reproduction of the unicellular green alga *Vitreochlamys aulata* (Pascher) Batko (= *Sphaerellopsis aulata* (Pascher) Gerloff) collected in Kanagawa Prefecture were observed in detail under controlled laboratory conditions. Although the vegetative morphology of the Japanese *V. aulata* material agreed with that described by Pascher (1927), Ettl (1963), and Batko (1970), the mode of flagellar formation in newly formed daughter cells differed from that observed by Batko (1970). This is the first report on *V. aulata* from Japan.

Key words: asexual reproduction, Chlorophyceae, morphology, *Shaerellopsis, Vitreochlamys aulata*

Vitreochlamys Batko (1970) (Chlamydomonadaceae, Volvocales, Chlorophyceae) is a unicellular green flagellate characterized by having a smooth, swollen cell wall separated from the protoplast (e.g., Ettl 1983). This taxon also appears in the literature under the name *Sphaerellopsis* Korschikov (1925) (e.g., Iyengar and Desikachary 1981, Ettl 1983, Nozaki 1991), a later homonym of the fungal genus *Sphaerellopsis* Cooke (1883). Batko (1970), therefore, proposed the nomen novum *Vitreochlamys* for encompassing the algal genus *Sphaerellopsis*.

Approximately twenty-three species of this genus have been described worldwide, mainly on the basis of differences in vegetative morphology (Ettl 1983). Recently, strains of *V. aulata* (Pascher) Batko were isolated from soils collected in Kanagawa Prefecture, Japan. This report describes the morphology and asexual reproduction of the Japanese strains of *V. aulata*.

Materials and Methods

Soil samples used in this study were collected from Tanasawa-paddyfields of Tokyo University of Agriculture, Shimokawairi, Atsugi-shi, Kanagawa Prefecture in March 1998. Clonal cultures (Spha-4/, Spha-5/, Spha-6/, and Spha-7/1998-3-9) were established by the pipette-washing method (Pringsheim 1946) from Petri dishes in which a small amount of the dried soil sample and a boiled pea (*Pisum sativum*) were rewetted with distilled water. The cultures were grown in screw-cap tubes (18×150 mm) containing about 12 ml of AF-6 medium (Kato 1982) modified by the elimination of $CaCO_3$ and by the addition of 400 mg/l MES, kept at 20°C, with alternating periods of 14 h light and 10 h dark at a light intensity of about 150 μmol/s/m^2 provided by cool-white fluorescent lamps. Observations of vegetative cells were carried out using actively growing, 5-day-old cultures which had originated from inoculations of cultures 4–6-days old. Light microscopy was carried out using an Olympus BX50 microscope equipped with Nomarski interference optics.

Observations

Vegetative cells were solitary, biflagellate and broadly ellipsoidal to obovoid in shape (Figs. 1, 2). The cell wall was swollen and separated from the surface of the protoplast (Figs. 1, 2, 4, 7). The protoplasts were subovoid to ellipsoidal with a pointed anterior end, sometimes developing a beak-like anterior structure. Flagella were as long as the cell length (Figs. 1, 7). Two or three contractile vacuoles were located near the base of the flagella (Figs. 5, 6). Chloroplasts were single and cup-shaped, with a single basal pyrenoid (Figs. 1b, 2), showing parallel longitudinal striations on the surface (Figs. 1a, 8). The stigma was single and nearly ellipsoidal, positioned near the middle of the cell (Figs. 1b, 3). The cells were 10–27 µm long and 9–22 µm wide. The protoplasts were 9–24 µm long and 9–

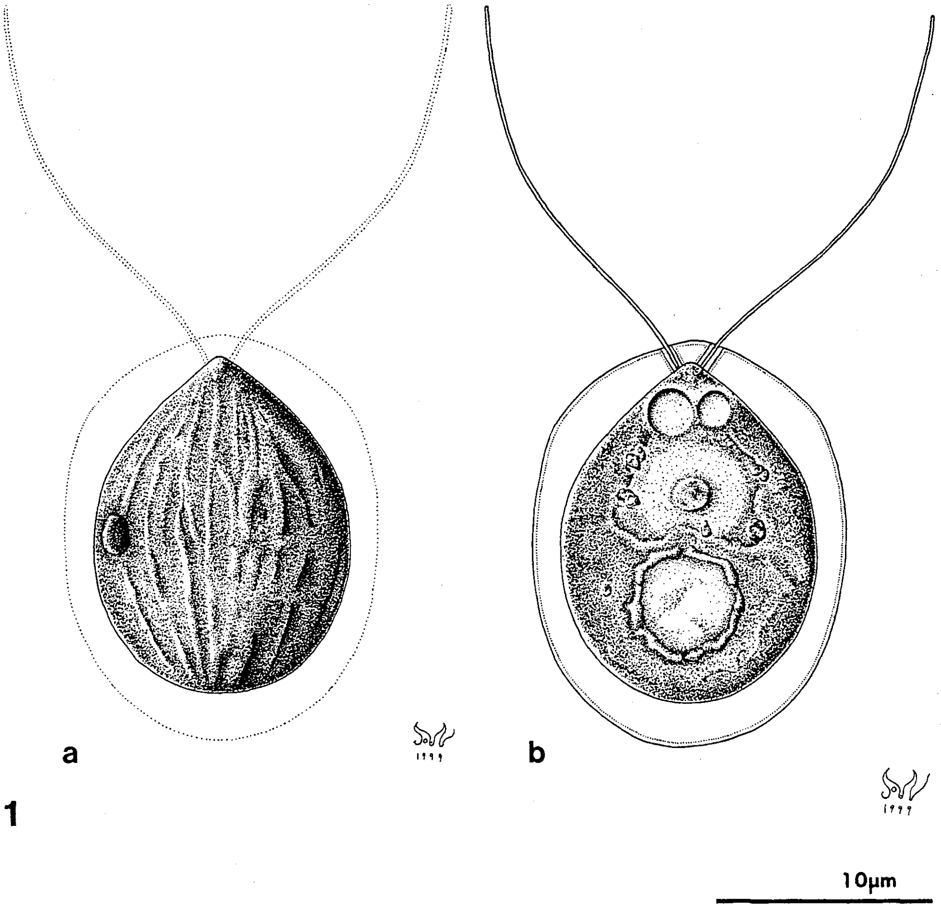

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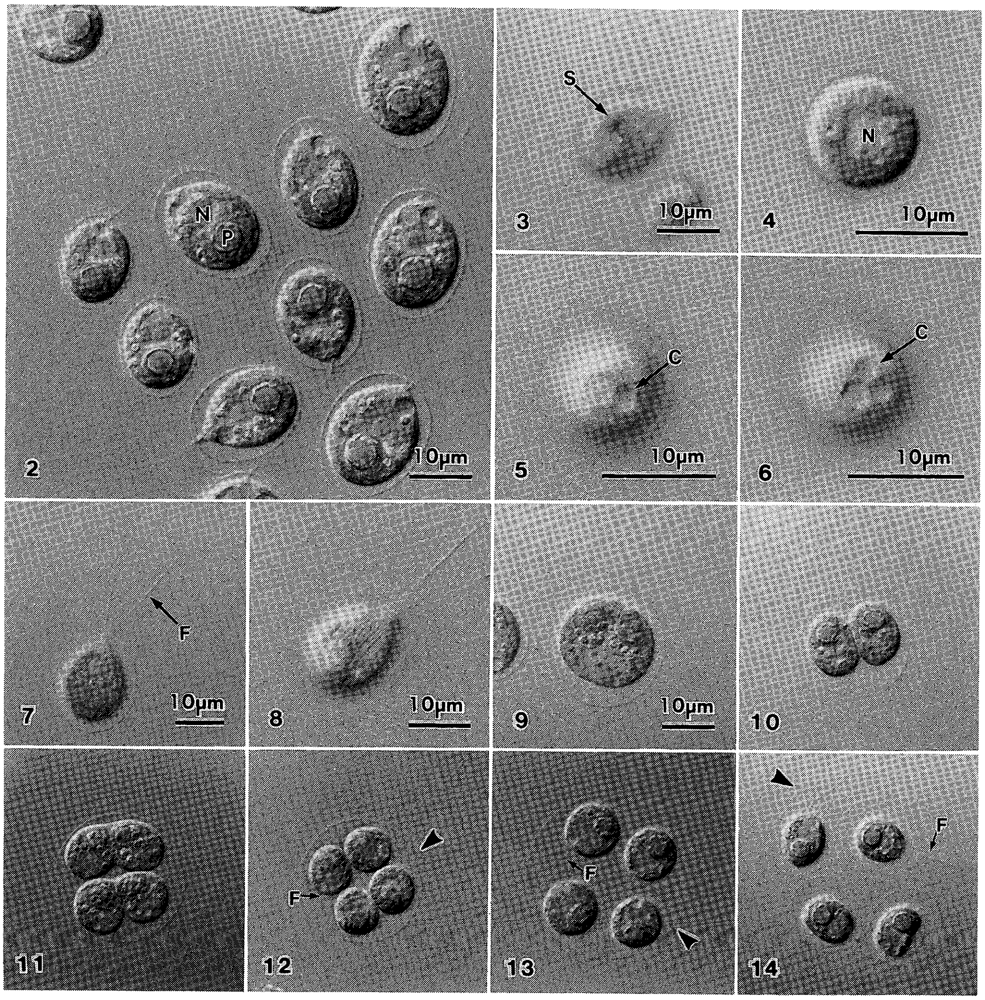
Fig. 1. Vegetative cell of *Vitreochlamys aulata* (Pascher) Batko, shown at the same magnification. a. Surface view. Note the longitudinal striations on the chloroplast. b. Optical section.



Figs. 2–13. Nomarski interference microscopy of the vegetative phase of *Vitreochlamys aulata* (Pascher) Batko. Fig. 2. Optical sections of cells, showing nucleus (N) and pyrenoid (P). Fig. 3. Surface view of cell, showing stigma (S). Fig. 4. Median transverse section of cell, showing nucleus (N). Fig. 5. Top view of cell, showing two contractile vacuoles (C) at the anterior pole of the protoplast. Fig. 6. Top view of cell, showing three contractile vacuoles (C) at the anterior pole of the protoplast. Fig. 7. Cell with two flagella (F). Fig. 8. Surface view of the protoplast, showing longitudinal striations on the chloroplast. Figs. 9–14. Asexual reproduction. All at same magnification. Figs. 9. First cell division. Note that the parental flagella have become detached from the cell. Fig. 10. Two-celled stage. Figs. 11. Second cell division. Fig. 12–14. Development of four daughter cells. Note new flagella (F) of daughter protoplasts developing within the parental cell wall (arrowheads).

18 μm wide. Asexual reproduction was accomplished by zoospore formation (Figs. 9–14). After the two flagella had become detached from the cell, the protoplast divided once (Figs. 9, 10), twice (Figs. 11–14) or three times successively to form two, four, or eight daughter protoplasts, respectively, within the parental cell wall. Each daughter

protoplast then grew two equal flagella and secreted a cell wall which soon became swollen (Fig. 14). The daughter cells were subsequently released from the parental cell wall.

Discussion

The vegetative morphology of the present Japanese alga agreed well with that of *Vitreochlamys aulata* reported by Pascher (1927), Ettl (1963) and Batko (1970) with regard to the single basal pyrenoid, the shape of the protoplast, and the parallel longitudinal striations on the surface of the chloroplast. Based on these features, *V. aulata* differs from other *Vitreochlamys* (*Sphaerellopsis*) species. In addition, vegetative cells of *V. aulata* sometimes show three contractile vacuoles near the base of the flagella (Ettl 1963, Batko 1970) (Fig. 6). This situation may be one of the morphological diagnoses of this species. However, some morphological differences were recognized between the Polish material of Batko (1970) and the present Japanese strains of *V. aulata*. According to Batko (1970), the cell was almost spherical and the flagella were 1½ times longer than the cell length (including the cell wall). On the other hand, the flagella in the present Japanese strains were nearly as long as the cell, and the cells were obovoid with a broad anterior face. Whether such morphological differences should be treated as species diagnoses or intraspecific variation remains to be determined on the basis of comparative morphology and/or molecular phylogenetic analysis using a large number of related strains of *Vitreochlamys* (see Nozaki et al. 1998).

Batko (1970) reported that in asexual reproduction of *Vitreochlamys aulata* each of the daughter protoplasts bears two equal flagella even when the first division of the parental protoplast is incomplete, and that these flagella of the daughter protoplast project through the parental cell wall until division is completed. In the present study, however, the parental cell lost its flagella just prior to the cell division (Fig. 9), and new flagella developed only after successive divisions of the protoplast within the parental cell wall (Figs. 11–14). This disagreement may have been due to the difference in the *Vitreochlamys* materials examined. However, development of the flagella of the newly formed daughter cells within the parental cell wall in the present Japanese strains is essentially the same as in other unicellular volvocalean species (see Ettl 1983).

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中沢 敦，野崎久義：日本新産種 *Vitreochlamys aulata* (緑藻綱，オオヒゲマワリ目）の形態と無性生殖

Vitreochlamys Batko (= *Sphaerellopsis* Korschikov) は単細胞性の淡水藻で，緑藻綱 (Chlorophyceae)，オオヒゲマワリ目 (Volvocales)，クラミドモナス科 (Chlamydomonadaceae) に分類されている．筆者等は最近神奈川県厚木市にある棚沢水田（東京農業大学）より日本新産種 *Vitreochlamys aulata* (Pascher) Batko を採取し，その栄養細胞の形態と無性生殖の過程を培養条件下で詳細に観察した．その結果，栄養細胞の形態は Pascher (1927), Ettl (1963) 及び Batko (1970) の記載と基本的に一致したが，無性生殖時における新しい鞭毛の形成様式が Batko (1970) のそれと異なった．

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